INFLUENCE OF AGE AND FEEDING PROGRAM ON SELECTED CHARACTERISTICS OF FOUR UNCOOKED BEEF MUSCLES

by

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TABLE OF CONTENTS

INTRODUCTION

Numerous studies have been made relating animal age to various characteristics of muscle, and generally it is agreed that the age of the beef animal at slaughter plays a major role in determining the eating quality. With economics of production causing changes in feeding patterns from previous years, more attention currently is being given to the effects of various feeding regimens on the quality of the meat produced.

It was the purpose of this study to compare selected characteristics of raw semimembranosus (SM), semitendinosus (ST), biceps femoris (BF), and longissimus dorsi (LD) muscles from 24 beef animals on 3 programs of feeding and slaughter age. More specifically, the objectives were:

1. To compare the effects of varying feeding programs and age of slaughter on the Warner-Bratzler shear value, percentage total moisture, water-holding capacity (WHC), pH, and color difference of selected raw beef muscles (SM, ST, BF, and LD).
2. To compare the effects of carcass location (3 muscles from the round and 1 from the loin) on the characteristics listed above.
3. To compare data for the histological estimate of fat and diameter of fibers in raw SM, ST, and BF muscles from animals of different age-feed levels.
4. To investigate relationships between the chemical and physical characteristics of the raw LD and organoleptic

evaluation of the cooked muscle from the same animals.

REVIEW OF LITERATURE

Effect of Selected Factors on Tenderness of Meat

Age of the animal. Tenderness is one of the most important sensory determinants of final eating acceptability of meat. It is also the primary characteristic of meat that is influenced by varying age of the animal. Most workers agree that tenderness decreases as animal age increases. Romans _et al_. (1965a) reported that LD steaks from mature carcasses were considered less tender by a taste panel than those from less mature carcasses. However, maturity had no significant effect on tenderness as determined by Warner-Bratzler shear. Henrickson and Moore (1965) found that taste panel scores showed a decrease in tenderness of beef as animal age increased from 18 to 90 months. Goll _et al_. (1963) stated that Warner-Bratzler shear force values of cores from BF steaks from the 3 oldest of 4 age groups of beef animals indicated that tenderness decreased with age. In another paper, Goll _et al_. (1965) stated that older animals were significantly less tender and received lower flavor intensity scores than younger animals. They also pointed out that wide extremes in carcass maturity influenced the eating quality of beef a great deal more than wide extremes in marbling.

Hiner and Hankins (1950) studied 9 beef muscles representing the principal wholesale cuts from animals of varying

age to determine their relative tenderness. Five age groups, from 10-week-old veal to 5½-year-old cows, were studied. As the age of the animal increased, tenderness decreased for each of the 9 muscles. Less difference in tenderness was found between the 2 youngest age groups than among the older, more mature animals.

Tuma _et al_. (1962a) also found that tenderness of LD steaks from animals 18, 42, and 90 months of age, as measured by both Warner-Bratzler shear and panel tenderness scores, decreased significantly ($P = 0.005$) with increasing animal age.

Nutrition of the animal. Graham _et al_. (1959) studied effects of both age and nutrition on characteristics of beef. Tenderness was not significantly affected by age in either drylot-fed or pastured animals. It was, however, affected by nutrition, increasing with increasing levels of nutrition in the drylot steers.

Several other studies were reported concerning the effect of nutrition of the animals on the tenderness of beef. Zinn _et al_. (1963b) placed 100 steers and 100 heifers on a growing-fattening ration. They slaughtered 10 steers and 10 heifers at the start (control) and every 30 days thereafter up to 270 days. Tenderness of the LD muscle increased significantly ($P = 0.05$) between the control animals and those on feed 150 days; however, no significant difference was observed after 270 days on feed. Kelly _et al_. (1963) fed rations with varying levels of protein to steers. Although the rate of efficiency

of gain was altered, cooking losses, tenderness (panel scores and Warner-Bratzler shear values), and juiciness of the meat were not consistently affected by the protein level. Jacobson and Fenton (1956a) also found no consistent effect of nutrition on shear force values. Meyer et al. (1960) studied the effects on palatability of grain-finished versus grass-finished beef. The panel scored the grain-finished beef significantly higher than the grass-finished for tenderness ($P = 0.05$), flavor of lean, flavor of fat, and juiciness ($P = 0.001$ for all). However, tenderness differences attributed to feeding regimes were not significant when measured by Warner-Bratzler shear.

Characteristics of muscle. Tenderness also has been related to various characteristics of the muscle. Blumer (1963) reviewed numerous studies concerning the relationship of marbling to the palatability of beef. Palatability was divided into 3 factors: tenderness, juiciness, and flavor. He summarized the several studies concerning the relationship of marbling to tenderness, and concluded that the variance in tenderness accountable to marbling would be about 5%. Blumer (1963) also estimated that approximately 16% of the variance in juiciness could be attributed to fat, and stated that the role of fat in flavor will be known only when the compounds of the flavor complex are all known in their proper proportions. Henrickson and Moore (1965) found tenderness varied with the fat level within muscle. The high fat level LD was most tender, followed by the high fat level ST, then the low fat level LD and ST.

Also, the histological evaluation of muscle fibers has been related to tenderness. Hiner et al. (1953) studied the relationship of fiber diameter in beef muscles to tenderness. The 9 cuts studied fell into 4 general groups according to increasing magnitude of fiber diameter as follows: (a) tenderloin; (b) 2 chuck samples, 8th rib, shortloin and loin end; (c) round; (d) neck and foreshank. There was little difference in diameter of the fibers from the 3 large muscles of the round, the SM, ST, and BF. Regression analysis showed the relationship between tenderness and fiber diameter to be curvilinear, the curvilinear correlation being 0.83. Analysis of variance showed that fiber diameter and tenderness attributed to animals and to samples was very highly significant, but the interaction of animals x samples was only significant.

Ramsbottom and Strandine (1949) described (with photomicrographs) the histological and tenderness changes as beef muscle passed through pre-rigor, rigor, and post-rigor periods. High shear readings were associated with wavy fibers in the rigor period, and with lower shear for the straighter fibers in the pre- and post-rigor periods. After 8-12 days, there was a gradual and progressive breakdown of the muscle fibers, and this histological observation was reflected in lower shear readings and higher tenderness ratings.

Effect of Age and Nutrition of the Animal on Selected Characteristics of Muscle

Fiber diameter. Romans et al. (1965a) noted a trend toward

larger fiber diameter for muscle fibers from more mature carcasses. This observation is corroborated by Hiner *et al.* (1953) and by Carpenter *et al.* (1963) who also noted increases in muscle fiber diameter with increasing animal age.

In a study by Lowe and Kastelic (1961) the smallest fiber diameter came from calves and, with one exception, the largest fibers came from the oldest animals. Fiber diameter was not correlated to tenderness of the meat, because the oldest animals were not the toughest.

Robertson and Baker (1933) studied the relationship of fiber diameter to level of feeding, and found that fiber diameter was largest in full-fed animals, intermediate for half-fed, and smallest for those on roughage only.

Color and pH. Color of muscle has been shown to be related to animal age or maturity. Jacobson and Fenton (1956b) noted a significant increase in redness (Hunter a/b ratio) of the SM muscle, both before and after cooking that was attributed to increasing age and increased level of nutrition of the animal. Similar trends of increasing redness with increasing level of nutrition were observed subjectively in the LD and psoas major muscles, but were not verified objectively. Fleming *et al.* (1960) attributed the color of fresh beef largely to the concentration and chemical state of myoglobin, and perhaps to some extent hemoglobin, which remains in the tissue as residual blood. Romans *et al.* (1965b) measured 3 components of color (Munsell hue, value, and chroma) for muscle from beef animals at 4 levels of maturity with a Photo-volt Reflection Meter.

Myoglobin and hemoglobin, on all 3 bases of analysis increased with advancing maturity, but only the differences between the youngest and the other 3 maturity levels were significant. Of the 3 Munsell color components only value was affected significantly ($P = 0.01$) by maturity, the differences being significant only between the youngest and other levels. Value tended to decrease with increasing maturity.

Wanderstock and Miller (1948) found only slight differences in color of lean between animals fattened on grass or grain or combinations of the two. They also reported that the same held true for pH, and Tuma _et al_. (1963) found that the pH of LD steaks was lowered with increased animal age.

Fat and moisture content. Both Wanderstock and Miller (1948) and Tuma _et al_. (1963) reported little difference in moisture content attributable to feeding program or animal age, except for a higher content in a 6-month-old calves than in older animals.

Jacobson and Fenton (1956a) observed that fat increased and moisture content decreased in raw muscle with an increase in level of nutrition of Holstein dairy cattle. With increase in age, in both raw and cooked meat, fat and shear force values increased. There was also a small but consistent decrease in moisture in the cooked meat with increase in age. Goll _et al_. (1963) also noted that veal had higher moisture and lower fat content than muscle from older animals. Zinn _et al_. (1963a) found a significant ($P = 0.05$) decrease in moisture in the SM, LD, and triceps as length of time on feed increased.

PROCEDURE

Twenty-four Angus steers sired by the same bull were selected at random from a group of 88 at approximately 6 months of age. Treatment varied with age-feed level at slaughter as follows: (1) 16 months, off winter roughage; (2) 20 months, off summer pasture; and (3) 25 months, off full feed. The animals were pastured in summer, roughed through winter (winter grazed), then 8 were slaughtered at 16 months of age, and the remaining 16 were placed on pasture. At 20 months of age, 8 were slaughtered off grass, and the remaining 8 placed on full feed until 25 months of age when they were slaughtered. Samples from SM, ST, BF, and LD muscles were removed from each carcass, wrapped, frozen at -10°F and held at 0°F. The frozen samples were thawed at room temperature to a semi-frozen state (approximately 45 to 75 minutes), the ends trimmed, the epimysium removed, and the sample divided for analysis as indicated by Fig. 1.

Chemical and Physical Measurements

<u>Warner-Bratzler shear values</u>. Two ½-inch cores were cut from each half of the sample, cutting with the grain of the tissue (Fig. 1). Two shears were made on each core with the Warner-Bratzler shearing apparatus, giving a total of 8 shear values per sample.

<u>pH</u>. Ground meat was packed into 100-ml glass beakers to a depth of approximately 1-in. and allowed to come to room

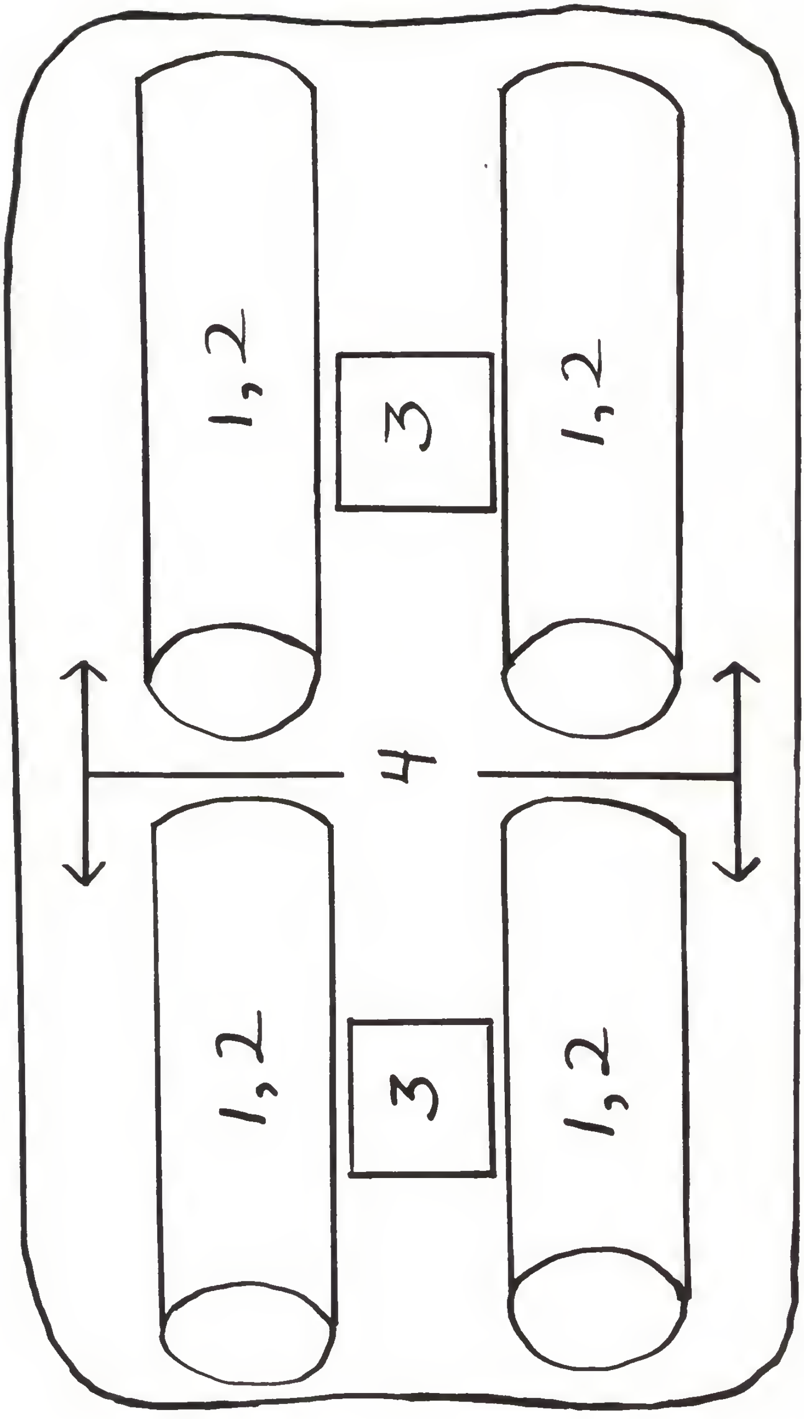
Fig. 1. Plan for sampling raw beef muscle sample.

1. Cores ($\frac{1}{2}$-in.) for shear value.

2. Water-holding capacity (the center portion of each core).

3. Cubes ($\frac{1}{2}$-in.) for histological specimans.

4. Total moisture, color difference and pH determined on samples of ground meat prepared from that remaining after the cubes for histological study were removed.


1,2
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temperature. Three pH readings were taken for each sample using a Beckman pH Meter (Model 76). A standard buffer of pH 6.86 was used to standardize the pH meter.

Color-difference. A Gardner color-difference meter was used to investigate color-differences in ground meat packed to a depth of at least 1-in. in an absorption cell with a glass bottom and plastic sides. Color-difference readings for Rd (reflectance), a+ (redness) and b+ (yellowness) were recorded for each sample. The instrument was standardized using a satin finish ceramic tile with calculated values as follows: Rd, 15.53; a+, 9.33; and b+, 13.10.

Total moisture. The percentage total moisture was determined by the use of the C. W. Brabender Semi-Automatic Moisture Tester. Duplicate 10-g samples of ground meat were weighed into calibrated dishes and subjected to a temperature of 121°F for 90 minutes.

Water-holding capacity. WHC was determined on the center portion of each core used for shear value (Fig. 1) by the procedure reported by Miller and Harrison (1965).

Histological Estimates

Specimens for histological estimates were removed from the interior of the samples from the SM, ST, and BF muscles between the cores that were taken for the shear measurements (Fig. 1). They were then fixed in physiological saline and formalin solution, and, in some cases, duplicate specimens were frozen.

Unfixed tissue was sectioned longitudinally at 8 u thickness, fixed tissue at 10 u thickness on a Model CTD--International-Harris Cryostat microtome, and stained to differentiate the muscle fibers and fat, leaving the connective tissue unstained. The stains used were Harris homotoxylin for the muscle fibers, Scarlet Red (Sudan IV) for fat. For details of staining procedure, see Appendix, p 40.

Five sections from each specimen were read by a panel of 3 using a Bausch and Lomb Dynazoom microscope. Estimates for quantity of fat were made according to a modification of the method described by Ramsbottom et al. (1945). The quantity of fat was estimated and given a numerical score according to the scale: large = 7, medium = 5, small = 3, none or trace = 1. The score for the specimen was an average of the scores for the 5 sections. For sample score sheet, see Appendix, p 41.

The width of 3 fibers in each section was measured (15 readings/person/specimen) with a calibrated ocular micrometer. For the detailed procedure, see Appendix, p 42. Also, relative width of muscle fibers was studied by counting the number of fibers in 1 to 3 fields at a specified magnification as described by Lowe and Kastelic (1961) with certain modifications. The modifications for this study were a magnification of 860x and a maximum of 3 fields per section in contrast to their magnification of 430x and 4 to 6 fields.

Statistical Analyses

Analysis of variance was conducted on data for each chemical and physical measurement to study the effect of the feeding program on selected muscle characteristics, and to determine differences among the muscles studied. When significant F-values were obtained, least significant differences at the 5% level were calculated. The analysis used when all 4 muscles were studied was:

Source of Variation	D/F
Muscle (R)	3
Treatment (S)	2
Muscle x treatment (RxS)	6
Remainder	83

For color-differences, when 3 of the muscles were studied, the analysis used was:

Source of Variation	D/F
Muscle (R)	2
Treatment (S)	2
Muscle x treatment (RxS)	4
Remainder	60

RESULTS AND DISCUSSION

The 16- and 20-month-old animals were slaughtered off winter roughage and summer pasture, respectively, and the 25-month-old animals were slaughtered off full feed. In the discussion of results, the treatments will be referred to by age or age-feed level.

Grade of Carcass

Effect of the feeding programs on USDA carcass grade is shown by the data in Table 1. Animals slaughtered at 16 months off winter roughage had the lowest carcass grades, mostly low standard and some high utility. Carcasses of 20-month animals were evenly divided between good and standard grades. Those graded good were either average or low good, and those graded standard were mostly high standard. The animals slaughtered off full-feed at 25 months were mostly choice animals with only one animal graded good. Carcass grades for animals in this study were in agreement with the report of Wanderstock and Miller (1948), who studied beef produced by yearling steers fed and managed according to methods ranging from entire dry-lot feeding to fattening solely on pasture. They stated that each year, the steers receiving no grain or other supplements

Table 1. Variation in carcass grade[a] of beef animals at 3 age and feed levels.

Treatment[b]					
16 mo		20 mo		25 mo	
No. of animals	%	No. of animals	%	No. of animals	%
6 S-	75 S	2 G		2 C+	
2 U+	25 U	2 G-	50 G	5 C-	87.5 C
		3 S+		1 G	12.5 C
		1 S	50 S		

a Grades, in descending order.
Prime (none in this study)
Choice = C
Food = G
Standard = S
Utility = U

b - 16 mo, slaughtered off winter roughage
20 mo, slaughtered off summer pasture
25 mo, slaughtered off full feed

had significantly lower final grades on foot and carcass grades than those of any of the other lots.

Chemical and Physical Measurements

Warner-Bratzler shear values. At all age levels, the LD shear value was significantly lower (more tender) than that for any of the other muscles. The relative tenderness (shear values) of the 3 muscles from the round varied with age. At 16 months the ST muscle was not significantly different from either the SM or the BF, but the SM and BF were significantly ($P = 0.05$) different from one another. At 20 months, the SM and ST were not significantly different, but the BF had a significantly ($P = 0.05$) lower shear value than either of the other 2 muscles. At those 2 age levels the BF was the most tender of the 3 muscles in the round. However, at 25 months the SM muscle rated significantly lower in shear force than the ST, and the lowest of the 3 muscles from the round, although not significantly lower than the BF (Table 2).

In general, when each muscle is compared against itself for the effect of the 3 treatments there was a decrease in shear value (increase in tenderness) with age and feed level. The BF muscle from 16-month-old animals required a significantly ($P = 0.05$) higher shear force than either the 20- or 25-month-old animals, but there was no significant difference between the 20- and 25-month-old groups. Shear force of the ST did not differ significantly between the 16- and 20-month-old

Table 2. Chemical and physical measurements for 4 muscles from beef animals at 3 age-feed levels.

Measurement	Treatment[a]	LSD[b] Muscle	LSD[b] Treatment	Muscle ST	Muscle SM	Muscle BF	Muscle LD
Shear							
lb/1/2 in. core	16 mo	0.865	0.749	10.9	11.2	10.2	6.0
	20 mo			10.8	10.0	8.9	7.2
	25 mo			9.0	7.7	8.2	4.0
pH							
	16 mo	0.032	0.028	5.80	5.69	5.73	5.74
	20 mo			5.72	5.66	5.69	5.68
	25 mo			5.70	5.65	5.71	5.63
Color							
Rd	16 mo	---	---	12.49	8.48	7.66	---
	20 mo			11.01	8.17	7.25	---
	25 mo			9.84	8.24	8.34	---
a+	16 mo	---	1.628	24.50	24.84	26.11	---
	20 mo			28.93	27.53	25.68	---
	25 mo			28.89	27.47	28.10	---
b+	16 mo	0.664	---	11.76	10.13	9.63	---
	20 mo			12.33	10.28	9.16	---
	25 mo			11.33	10.34	11.02	---
Total moisture, %							
	16 mo	0.723	0.626	76.22	75.90	76.10	74.89
	20 mo			75.43	74.97	75.57	74.19
	25 mo			73.32	72.03	73.05	70.03
Water-holding capacity							
	16 mo	---	0.042	0.36	0.33	0.30	0.33
	20 mo			0.35	0.39	0.39	0.39
	25 mo			0.35	0.22	0.24	0.27

a - 16 mo, slaughtered off winter roughage
20 mo, slaughtered off summer pasture
25 mo, slaughtered off full feed

b -LSD, least significant difference at the 5% level

animals, but there was a significant (P = 0.05) decrease from 20 to 25 months. The SM decreased significantly (P = 0.05) in shear with each increase in age and feed level. The LD also differed significantly (P = 0.05) among treatments, but for this muscle there was an increase in shear force between 16 and 20 months with a subsequent decrease between 20 and 25 months. Henrickson and Mjoseth (1964) noted that the ST muscle is more uniform in tenderness than the LD and thus, may be more suitable for studies designed to demonstrate tenderness differences resulting from treatment. This may explain, in part, the significant decrease in tenderness of the LD between 16 and 20 months followed by a significant increase between 20 and 25 months. There was an over-all decrease in shear force of the LD between 16 and 25 months that was significant (P = 0.05). The SM muscle decreased the most in shear with increased age and feed level, going from 11.2 lb at 16 months (highest value for the 3 muscles from the round) to 7.7 pounds at 25 months (the lowest value for the 3 muscles from the round). (Table 2)

Most workers have found that tenderness decreases with age; however, in this study all 4 muscles became more tender as slaughter age increased. Thus, it appears that the feeding program had greater effect on tenderness than age of the animal. There were no consistent effect differences in tenderness among muscles for the animals slaughtered at 16 months off winter roughage and those slaughtered at 20 months off summer pasture. Two of the muscles (BF and SM) increased in tenderness, one (ST) did not change and one (LD) decreased in tenderness

between 16 and 20 months. On the other hand, only one muscle (BF) failed to improve significantly (P = 0.05) in tenderness between 20 and 25 months (summer grazed versus full feed) (Table 2). It seems reasonable to assume that animals on full feed are obtaining a higher level of nutrition than those on summer pasture or winter roughage. Thus, there was an increase in tenderness, as shown by Warner-Bratzler shear force values, as level of nutrition increased in spite of the fact that age also increased. This is in contrast to Meyer _et al_. (1960), who found no significant differences in tenderness attributable to feeding regimes (grain-finished versus grass-finished beef) when tenderness was measured by Warner-Bratzler shear. However, the grain-finished beef was scored significantly higher in tenderness by a taste panel.

_pH_. The ST muscle from 16-month-old animals had a significantly (P = 0.05) higher pH than any of the other muscles, whereas the SM was significantly (P = 0.05) lower in pH than any of the others. At 20 months, the ST was significantly (P = 0.05) higher in pH than the LD or the SM, but no other significant differences were observed. At 25 months, the ST and BF muscles were both significantly (P = 0.05) higher in pH than either the SM or LD. There were no significant differences between ST and BF or between SM and LD. Thus, the ST tended to have the highest pH of the 4 muscles and this was most evident in the lowest age group. The BF muscle, as compared to the other muscles, tended to increase in pH with age and feed level until for the 25-month-old animals it was not significantly different from the ST (Table 2).

The LD decreased significantly ($P = 0.05$) in pH between each age and feed level. The other 3 muscles decreased significantly ($P = 0.05$) in pH between the 16- and 20-month levels, but no significant change occurred between the 20- and 25-month levels. All but the BF muscle decreased significantly ($P = 0.05$) in pH between the 16 and 25 month groups (Table 2).

Wanderstock and Miller (1948) found only slight differences in pH between animals fattened on grass or grain or combinations of the two. Tuma _et al_. (1963) found that the pH of LD steaks decreased with increased animal age.

Color-difference. _Rd_, measured by the Gardner color-difference meter, is the reflectance value of the sample, which is the amount of light reflected by the sample as opposed to the amount of light transmitted or diffused. A completely absorbing sample (dark or opaque rather than light or clear) would have an _Rd_ value of zero, whereas a perfectly diffusing white would have an _Rd_ of 100. The SM and BF muscles were not significantly different in reflectance from one another, but the ST was significantly lighter (whiter) than the other 2 muscles at all age levels. The BF became significantly lighter between 20 and 25 months. The ST was the only muscle that changed consistently with the treatment, becoming darker with age and feed level, as evidenced by lower _Rd_ values (Table 2). This is in agreement with Romans _et al_. (1965b) who found that Munsell value (lightness) decreased with maturity.

Gardner color-difference meter values of _a_ denote redness

or greenness, a+ being red, whereas a- is green. Only a+ values were obtained for muscles evaluated in this study. A value of zero for either a or b components indicates some shade of gray. There were no significant differences among muscles for the red component. All the muscles were approximately the same redness and all increased in redness with increased age and feed level. Both the ST and SM muscles increased significantly ($P = 0.05$) in a+ values (increased in redness) between 16 and 20 months, but not between 20 and 25 months. The BF, on the other hand, increased significantly ($P = 0.05$) in redness from 20 to 25 months, but not between 16 and 20 months. All 3 muscles increased significantly ($P = 0.05$) in redness between 16 and 25 months (Table 2).

Whereas Wanderstock and Miller (1948) found only slight differences in color of muscle from grass- and grain-fattened animals, Jacobson and Fenton (1956b) found a significant increase in redness with increasing age and increasing level of nutrition. This latter study is in agreement with the results reported here.

Gardner values of b+ measure yellowness of a sample, whereas b- denotes blueness. The ST muscle tended to be more yellow than the SM or BF muscles. At 16 and 20 months it was significantly ($P = 0.05$) more yellow than either of the other muscles (Table 2). The BF muscle at 25 months was significantly ($P = 0.05$) more yellow than at either the 16- or the 20-month levels.

Total moisture. Percentage total moisture was measured by the C. W. Brabender semi-automatic moisture tester. A comparison of the moisture levels of the different muscles indicated the LD contained significantly ($P = 0.05$) less moisture than all the other muscles at all age levels. There were no significant differences among the 3 muscles from the round (SM, ST, and BF) at the 16- or 20-month levels. At 25 months, however, the SM was significantly ($P = 0.05$) lower in moisture than the ST or BF. There were significant ($P = 0.05$) decreases in total moisture between the 16 and 20 month groups for all muscles except BF and between the 20 and 25 month groups for all muscles (Table 2).

Data reported by other workers differ concerning moisture content of beef muscle as influenced by age and nutrition of the animal. Neither Wanderstock and Miller (1948) nor Tuma et al. (1963) found significant differences in moisture content that were attributable to either age or feeding program. On the other hand, Jacobson and Fenton (1956a) and Zinn et al. (1963a) found variations in moisture content with different levels of nutrition. Moisture decreased as level of nutrition or time on feed increased. According to Goll et al. (1963), veal had a higher moisture content than muscle from older animals, but there was no indication of differences among mature animals of different ages. With increase in age, Jacobson and Fenton (1956a) found a consistent, but small, decrease in moisture in cooked meat but not in raw. Thus, it seems that nutrition of the animal has a more definite effect on moisture

content than does age, moisture decreasing with increasing level of nutrition. These observations were confirmed by this study.

Water-holding capacity. The ratio of the area of a pressed meat sample to the area of expressed liquid formed on filter paper on which the sample was pressed was designated as expressible-liquid index by Miller and Harrison (1965). They obtained values for WHC by subtracting the expressible-liquid index from one, which arbitrarily was chosen as the maximum expressible-liquid index. In this study, data for WHC were obtained in the same manner. Since the magnitude of the expressible-liquid index is inversely related to the amount of liquid expressed from the sample, the larger the value for WHC, the greater the amount of liquid expressed.

There were no significant differences in WHC among muscles, and the ST muscle did not vary significantly among treatments. For the other 3 muscles (SM, BF, and LD), however, there was a significant ($P = 0.05$) increase in WHC between 16 and 20 months, then a significant decrease between 20 and 25 months with a decrease from 16 to 25 months that was significant. These 3 muscles all had the same value for WHC at 20 months (Table 2).

Histological Estimates

Quantity of fat. Generally the estimated quantity of fat in all muscles, as might be expected, increased with increases in age and nutrition of the animal. With only one exception (unfixed ST, 16 to 20 months), a definite and substantial

increase in fat level was observed. For unfixed tissue, the SM and BF followed similar trends. The fat in the SM muscle was at a slightly lower level at 16 months, but at 20 and 25 months the fat levels in the 2 muscles were nearly the same. Values for the unfixed ST specimens increased the least with increased age and feed level, being higher than unfixed specimens from the SM and BF at 16 months, and lower at 25 months. The estimated quantity of fat in fixed tissue also increased at every age and feed level, but, in this case, the ST increased more than the SM muscle. All the specimens (fixed and unfixed) contained a "small" to a "medium" quantity of fat as judged subjectively (Table 3).

The differences between fixed and unfixed sections in quantity of fat did not follow a consistent trend, and were no more than might be expected for 2 blocks of tissue within a muscle. Lowe (1948) pointed out the limitations of histological study by stating that any microscopic section under observation represents a small area of the entire muscle, and that if several sections are made from different areas of a sample of muscle they may vary greatly. Further, in the same section, different areas may vary widely. Van Hulle _et al_. (1965) studied how accurately the void spaces in histological sections represent the size and location of ice crystals in frozen material. They concluded that while some of the finer structures of the material may be altered, the fixing and staining procedures used did not result in any gross misrepresentations of the frozen structure, and that the results

Table 3. Histological evaluation of 3 raw muscles from the round of beef animals at 3 age-feed levels.

Treatment[a]		Muscle SM		Muscle ST		Muscle BF	
		Quantity of fat[b]					
Unfixed	16 mo	2.7 (4)[c]		3.8 (4)		3.4 (1)	
	20 mo	4.0 (7)		3.6 (7)		4.0	
	25 mo	5.0		4.2		5.1	
Fixed	16 mo	3.1		2.9		---	
	20 mo	3.8		3.7 (7)		---	
	25 mo	4.3		5.2		---	
		Muscle fiber diameter					
		u	No.[d]	u	No.	u	No.
Unfixed	16 mo	30.0	5.9 (4)	26.4	8.2 (4)	30.2	5.5 (1)
	20 mo	32.0	5.3 (7)	27.7	5.8 (7)	28.9	5.6
	25 mo	36.9	4.9	33.1	5.3	31.2	5.3
Fixed	16 mo	35.4	5.2	38.5	4.8	---	---
	20 mo	37.7	4.9	36.3	4.7 (7)	---	---
	25 mo	45.3	4.2	45.3	4.2	---	---

a Unfixed - frozen storage
Fixed - formalin and physiological salt solution
16 mo - slaughtered off winter roughage
20 mo - slaughtered off summer pasture
25 mo - slaughtered off full feed

b Quantity of fat
large = 7
medium = 5
small = 3
none or trace = 1

c - Numbers in parentheses indicate the number of specimens in the group. Unless otherwise indicated, each average represent 8 specimens.

d - No. - number of fibers in microscopic field at a magnification of 860x.

failed to provide any evidence which would justify criticism of the technique.

Histological estimate for quantity of fat in this study are in agreement with those reported by other workers who measured fat by ether extract. Jacobson and Fenton (1956a) found that fat in raw muscle increased with age and with nutrition of the animal. Goll et al. (1963) also found that young animals (veal) had a lower fat content than more mature animals.

Figure 2 (a and b) shows the differences in quantity of fat in the ST muscle between the animals slaughtered at 16 months off winter roughage and those slaughtered at 25 months off full feed. Picture a is of the ST muscle of one of the animals slaughtered at 25 months. The top 1/3 to ½ of the picture is a portion of a large fat area that ran throughout the section. The average score for fat content of the ST muscle for this animal was 6.3 A small quantity of fat can be seen in the center of picture b (dark spots and the small gray areas surrounding them), which represents muscle from a 16-month steer. The average score for fat content for the ST muscle of this animal was 2.9.

Muscle fiber diameter. Muscle fiber diameter was measured by 2 methods: (1) by the measurement, in microns, of the width of the fiber by a calibrated ocular micrometer, and (2) by counting the number of fibers in a microscopic field at a specified magnification. By the latter method, the larger the number of fibers in the field, the smaller the fiber diameter.

Figure 2. Photomicrographs of muscle from beef animals at 2 age and feed levels with the samples preserved by 2 methods (mag = 200x)

a. ST muscle from animal 82, slaughtered at 25 months off full feed. Sample preserved by fixation in formalin and physiological salt solution.

b. ST muscle from animal 68, slaughtered at 16 months off winter roughage. Sample preserved by fixation in formalin and physiological salt solution.

c. ST muscle from animal 85, slaughtered at 25 months off full feed. Sample preserved by fixation in formalin and physiological salt solution.

d. ST muscle from animal 85, slaughtered at 25 months off full feed. Sample preserved by freezing.

<u>a</u> and <u>b</u> show differences in fat content with age and feed level.

<u>c</u> and <u>d</u> show differences in fiber diameter with preservation method.

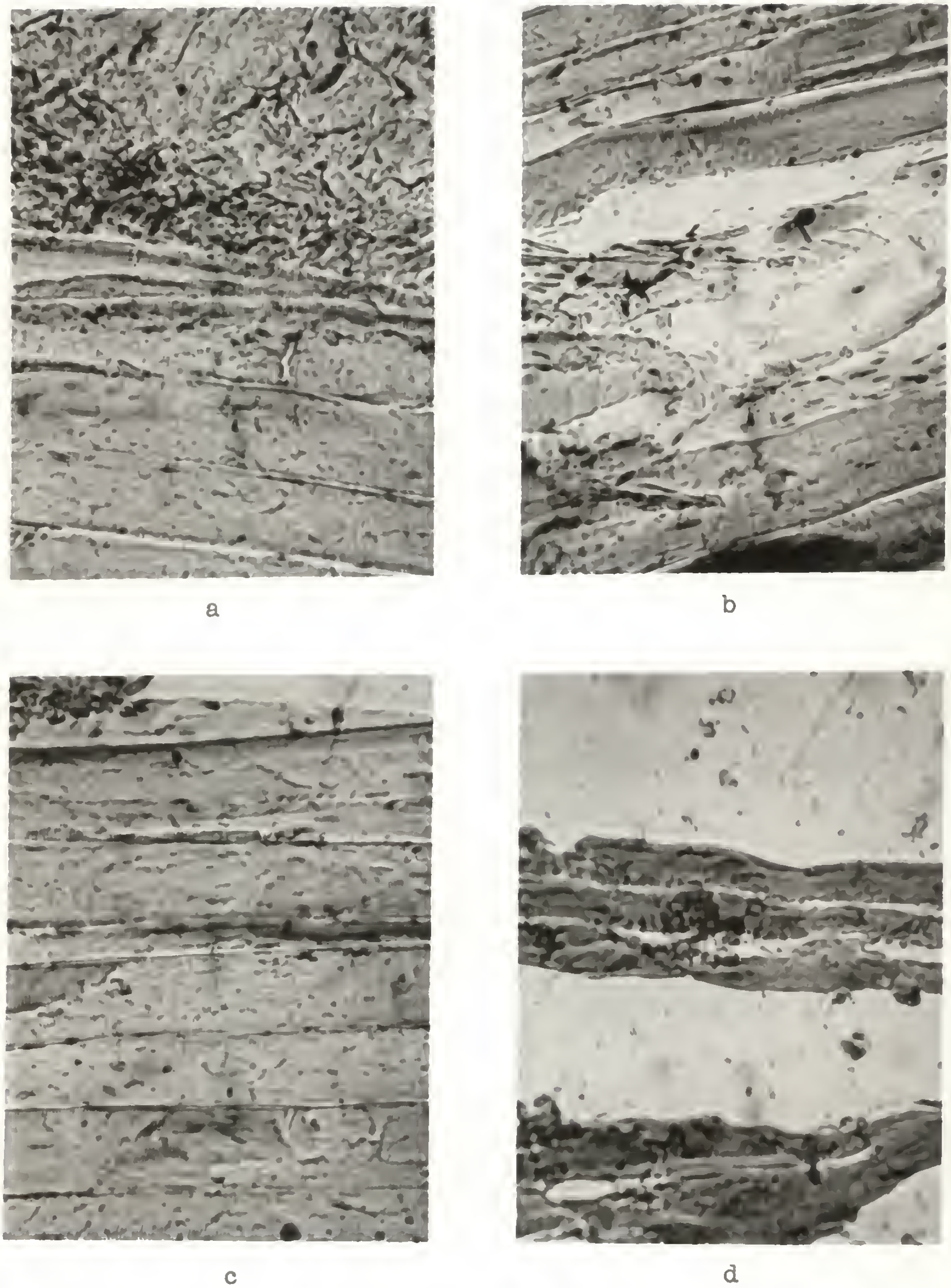

Fig. 2. Photomicrographs of muscle from beef animals at 2 age and feed levels with the samples preserved by 2 methods. (mag = 200x)

Thus, the 2 methods of measurement produce data that vary inversely (Table 3).

Muscle fibers in unfixed SM and ST, and fixed SM increased in diameter with age and feed level. The measurement in microns increased consistently, whereas the number of fibers in the field consistently decreased with treatment. The micron measurement for the unfixed BF and the fixed ST indicated that muscle fiber diameter decreased slightly (16 to 20 months), then increased (20 to 25 months). The increase was slight for the unfixed BF and substantial for the fixed ST sample. The number of fibers in the field for these 2 muscles remained relatively unchanged between 16 and 20 months, and then decreased slightly between 20 and 25 months. The fiber diameter ranged from approximately 25 to 45 μ, with 2/3 of the specimens falling in the 30's (Table 3).

The unfixed muscle fibers were, in all cases, smaller in diameter than the corresponding fixed samples (Table 3). Differences in average fiber diameter between fixed and unfixed specimens from animals in the same age and feed group ranged from 5.4 to 12.2 μ. These differences were between sections from the same muscle sample, varying only in preservation method. The histological specimens were taken from the semi-frozen muscle, and placed either in the fixative solution (fixed) or wrapped in aluminum foil and placed back into frozen storage (unfixed) until sectioning.

Pictures c and d in Figure 2 are sections from the same muscle and the same animal. The specimen in picture c was

preserved by fixation; the one in picture d was preserved by freezing. The average fiber diameters for the fixed and frozen specimens (animal 85, ST muscle) were 40.2 μ and 28.7 μ with 4.5 and 6.0 as the average numbers of fibers in a high-power microscopic field, respectively.

Two explanations for the smaller diameter of fibers in the unfixed sections seem feasable. Either the fixative solution caused a slight swelling of fibers, or the frozen storage caused shrinkage of fibers. Carpenter et al. (1963) used 3 fixatives, 10% formalin, 1.5% picric acid, and 1% osmic acid, in their study of histological characteristics of pork muscle. Picric acid was preferred by the workers, because it produced less shrinkage of tissue than did the formalin. Thus, it would not seem likely that in this study formalin and physiological salt solution would produce swelling of tissue. It is possible that the wrapping of frozen specimens was not adequate to prevent dehydration of the tissue in the freezer which produced a shrinkage of fibers.

Fiber diameter often is related inversely to the tenderness of the meat, tenderness decreasing as fiber diameter increases. Tuma et al. (1962b) stated that the effect fiber diameter may have on tenderness appears to be attributable to the animal age-fiber diameter relationship. This means that fiber diameter and tenderness are related to one another, because each is related to age of the animal. In this study, however, although fiber diameter increased with age as expected, the tenderness also increased. So, in this instance, tenderness and fiber diameter were related positively.

Carpenter et al. (1963) noted that there was a positive, but non-significant relation between fiber diameter and intramuscular fat. The results of this study also show a tendency for fiber diameter and quantity of fat to increase together.

Palatability and Cooking Data for Rib Roasts

Palatability and cooking data were available for rib roasts (left ribs 6-8) from the same animals used for chemical and physical measurements of the LD (Table 4). Ribs were roasted at 300°F to an internal temperature of 158°F, and both subjective and objective measurements related to palatability were made on samples from the LD, whereas cooking losses and time were determined for the entire roast.

Tenderness. The palatability panel scored tenderness according to both initial impression and the number of chews required to masticate a ½-in. cube of meat. The initial tenderness scores indicated essentially no change in tenderness from 16 to 20 months with an increase in score, and in tenderness, from 20 to 25 months. The score based on chews was lower than the initial tenderness score at 16 months and increased consistently with age and feed level. At 20 and 25 months the 2 subjective scores were essentially the same.

The objective measurement of tenderness (Warner-Bratzler shear force) was taken on a 1-in. core of cooked LD muscle. Shear force values were essentially the same from 16 to 20 months, then decreased from 20 to 25 months indicating an increase in tenderness. This is the same trend observed in

Table 4. Objective and subjective evaluation of cooked rib roasts (left ribs 6-8) from beef animals at 3 age-feed levels.[a]

Measurement	Treatment[b] 16 mo	20 mo	25 mo
Tenderness			
Initial score[c]	4.8	4.9	6.0
Chew score[c]	4.5	4.8	6.0
Shear value (lb/1-in. core)	17.0	17.7	15.4
Flavor[c]			
of fat	---[d]	4.4	5.7
of lean	4.5	5.0	5.9
Juiciness			
Score[c]	5.6	5.7	5.3
Press fluid (ml/25 g)	9.3	7.5	6.4
Cooking losses (%)			
Total	18.4	13.6	25.3
Volatile	15.2	11.0	18.1
Dripping	3.2	2.6	7.2
Cooking time (min/lb)	39.3	33.1	32.9

a - Unpublished data, Department of Foods and Nutrition.
b -16 mo, slaughtered off winter roughage
20 mo, slaughtered off summer pasture
25 mo, slaughtered off full feed
c - Maximum possible score, 7
d - Too little fat on the roasts to provide palatability samples

the initial tenderness scores of the panel, but is not the same pattern followed by the shear force values for a ½-in. core of the raw LD muscle (Table 2).

<u>Flavor and juiciness</u>. Flavor scores for both fat and lean increased consistently with age and feed level, whereas juiciness scores were not affected by treatment. Meyer *et al.* (1960) reported that grain-finished beef was scored superior to grass-finished beef for flavor of fat and lean and for juiciness. Differences in juiciness between the 2 types of beef were significant. Henrickson and Moore (1965) found no significant differences in panel scores for juiciness attributable to age of the animal.

Press fluid is the volume (ml/25 g) of fluid pressed from ground cooked meat under a specified time-pressure schedule. Press fluid decreased substantially and consistently with increased age and feed level (Table 4). This trend corresponds to the significant ($P = 0.05$) decreases in percentage total moisture in the raw meat (Table 2). Wanderstock and Miller (1948) found only slight differences in moisture content and in press fluid among animals fed on grain or pasture or some combination of the two. Sanderson and Vail (1963) found that both total moisture content and press fluid decreased with increased internal end-point temperature of cooked meat.

Cooking losses. Dripping, volatile, and total cooking losses all followed the same general trend. For total losses there was a definite decrease of approximately 5% from 16 to 20 months followed by an increase of almost 12% between 20 and 25 months. Graham *et al*. (1959) noted significant ($P = 0.01$) differences in total cooking losses and juiciness attributable to nutrition and age in drylot-fed steers. In pastured steers, total cooking losses increased with age, but were not affected by previous drylot nutrition. Kelly *et al*. (1963) found no consistent effect of protein level of the animal ration on cooking losses, juiciness or tenderness (as measured by panel and Warner-Bratzler shear) of the meat. This decrease, then increase pattern is the same as that observed for tenderness as measured objectively in the raw muscle, and is opposite of WHC of the LD which increased, then decreased with age and feed level.

Cooking time. Cooking time (min/lb) also varied with age and feed level. There was a sharp decrease from 16 to 20 months with the values for 20 and 25 months approximately the same. Possible reasons for the differences in cooking time may be differences in the weight and fat content and cover of the roast.

SUMMARY

Selected muscles (SM, ST, BF, LD) from 24 half-sibling steers at 3 age and feed levels were studied for differences in certain chemical, physical, and histological characteristics. Data for physical and chemical measurements on the LD were discussed in relation to palatability and cooking data available for rib roasts from the same animals. Eight animals were slaughtered and the carcasses graded at each age and feed level: (1) 16 months, off winter roughage; (2) 20 months, off summer pasture; and (3) 25 months, off full feed.

Warner-Bratzler shear values for a ½-in. core of raw muscle indicated that the LD was the most tender of the muscles studied. In general, there was an increase in tenderness (decrease in shear) with increasing age and feed level.

The ST tended to have the highest pH of all 4 muscles, which was most evident in the 2 lower age groups. For 3 muscles, there was a decrease in pH with increased age and feed level.

Gardner color-difference measurements (Reflectance, Rd; redness, a+; yellowness, b+) for SM, ST, and BF indicated

that the ST was significantly (P = 0.05) lighter in color than the other 2 muscles, and it was the only one that changed consistently with treatment, becoming darker with increased age and feed level. It was also more yellow than the other 2 muscles. All 3 muscles increased significantly (P = 0.05) in redness between 16 and 25 months.

In general, there was little difference in percentage total moisture among the 3 muscles from the round, but at all age levels the LD contained significantly (P = 0.05) less moisture than any of the muscles from the round. Total moisture tended to decrease in all 4 muscles with increased age and feed level.

Irrespective of age and feed level, values for WHC were similar for all muscles. However, WHC values for all muscles, except ST, increased significantly (P = 0.05) between 16 and 20 months, then decreased significantly (P = 0.05) between 20 and 25 months.

Histological evaluation of SM, ST, and BF indicated that the estimated quantity of fat and diameter of muscle fiber increased with increased age and feed level. Muscle fibers in specimens preserved by freezing were consistently and substantially narrower than fibers in specimens fixed in formalin and physiological salt solution.

Carcass grade of the animals, and tenderness and desirability of the flavor of rib roasts tended to increase with increased age and feed level. Juiciness scores for rib roasts were similar at all age and feed levels, whereas press fluid

decreased substantially with each increase in age and feed level. The decrease in press fluid corresponded to the pattern for percentage total moisture in the raw LD.

Cooking losses followed an increase--decrease pattern, which was opposite to the pattern of WHC values for raw LD. Cooking time decreased with increased age and feed level.

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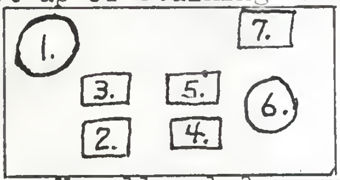
APPENDIX

STAINING AND MOUNTING PROCEDURES[a]

The cut section was picked up from the knife blade by pressing a slide against the frozen meat section. The sections were cut, picked up and left exposed to air (room temperature) until a group of 9-10 slides was attained. The staining procedure was as follows:

1. water, tap - dip
2. stain, hemotoxylin - for a period of $1\frac{1}{2}$-2 minutes
3. water, tap - rinse
4. stain, Sudan IV (Scarlet Red) - for a period of 1-$1\frac{1}{2}$ minutes
5. alcohol, 70% - rinse
6. water, tap - rinse
7. water, tap - storage until mounting

Set-up of staining dishes:



Usually, 1-3 groups of slides were stained before mounting was begun. The slides were cleaned with a dry cloth, care being taken to avoid damage to the meat section. One or two drops of glycerine jelly (heated in a hot-water bath) were placed on the section and a cover slip, cleaned in 95% alcohol and dried with a soft cloth, was placed over the meat area. The glycerine jelly was allowed to cool and solidify. The slides were then observed under the microscope and 5 were chosen from each group for study by the panel.

[a]adapted from "Microtome-Cryostat Handbook," International Equipment Co., Needham Heights, Mass. 1964.

Histological Sample Score Sheet

Raw Beef Muscles

Code no. ______________ Name ____________________

	Slide number 1	2	3	4	5	Total	Average
Relative quantity of fat[a]							
Muscle fiber diameter (mm, 200x) a)							
b)							
c)							
Total							
No. in high power field (860x) (if possible) a)							
b)							
c)							
Total							

[a]Quantity Score
- Large - 7
- Medium - 5
- Small - 3
- None - 1

Procedure for Measuring Diameter of Muscle Fibers

Calibration of Micrometer

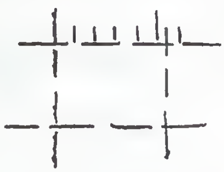
Insert ocular micrometer (the round one) in eyepiece of the miscroscope.

Insert stage micrometer (the slide) on the stage of the microscope.

Using low power (10x eyepiece and 10x objective, zoom setting 2 = 200x magnification) find the scale and bring it into focus.

Match a line of the scale on the stage micrometer with a line on the squared scale of the ocular (eyepiece) micrometer.

Example:

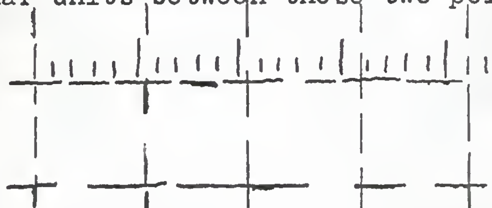


stage scale

squared ocular scale

Look further down the scale and find another point at which the lines match, count the number of stage units and the number of ocular units between these two points.

Example:



21 stage units

= 4 ocular units

Give the distance covered by the stage units its numerical value (each stage unit = 0.01 mm, see slide). Divide this value by the number of ocular units, thus finding the value of each ocular unit.

Example:

21 stage units = 21 x 0.01 mm or 0.21 mm

$$\frac{0.21 \text{ mm}}{4 \text{ ocular units}} = 0.0525 \text{ mm/ocular unit (1 ocular unit=0.0525 mm)}$$

Measuring Fiber Diameter

Remove the stage micrometer and replace it with the slide being studied. At random, select 3 fibers from the section of tissue on the slide, and measure the width of each fiber. Measurements of the muscle fibers can be made using the values assigned to the ocular units. Convert values in mm to μ.

Note: These values are good only as long as low power is used. If high power is to be used for measurement, the high power objective should be used for calibration.

Note: Through the center of the eyepiece, the ocular units are divided into fifths. These should be used in measurement for greater accuracy.

Note: The eyepiece can be turned in the tube, thus turning the lines of the ocular scale. In this way, fibers can be measured even though they do not lie in a perfectly vertical or horizontal direction.

INFLUENCE OF AGE AND FEEDING PROGRAM ON SELECTED CHARACTERISTICS OF FOUR UNCOOKED BEEF MUSCLES

by

KAROLYN LINDER ELY

B. S., Kansas State University, 1965

AN ABSTRACT OF A THESIS

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1967

Semimembranosus (SM), semitendinosus (ST), biceps femoris (BF), and longissimus dorsi (LD) muscles from 24 half-sibling steers at 3 age and feed levels were studied for differences in certain chemical, physical, and histological characteristics. Data for physical and chemical measurements on the LD were discussed in relation to palatability and cooking data available for rib roasts from the same animals. Eight animals were slaughtered and the carcasses graded at each age and feed level: (1) 16 months, off winter roughage; (2) 20 months, off summer pasture; and (3) 25 months, off full feed.

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Cooking losses followed an increase--decrease pattern, which was opposite to the pattern of WHC values for raw LD. Cooking time decreased with increased age and feed level.